Blockade by mazindol of brain 5-HT depletion induced by *p*-chloromethamphetamine*

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Mazindol (5-hydroxy-5-*p*-chlorophenyl-2,3-dihydro-5H-imidazo-2,1-α-isoindole), a potent anorexigenic agent (Zambotti, Carruba & others, 1976), has been shown previously to inhibit the high-affinity uptake of labelled 5-hydroxytryptamine (5-HT) by rat (Buczko, de Gaetano & Garattini, 1975) and guinea-pig (Picotti, Carruba & others, 1977) blood platelets *in vitro*. It has been suggested that it competes with 5-HT for the same transport mechanism at the cytoplasmic membrane (Picotti & others, 1977). The inhibition of 5-HT uptake in rat forebrain synaptosomes has also been demonstrated *in vitro* (Carruba, Picotti & others, 1977). Moreover, synaptosomes isolated from the forebrain of rats 1 h after the injection of different doses of mazindol showed a marked reduction in the 5-HT uptake compared with synaptosomes from the forebrains of untreated animals. A dose as low as 2·1 μmol kg^{-1} had a significant effect while 35 μmol kg^{-1} inhibited uptake by approximately 45%. In the same experimental conditions chlorimipramine, another inhibitor of 5-HT uptake (Carlsson, Corrodi & others, 1969), at low doses was less effective, while when given at 35 μmol kg^{-1} it showed a more pronounced effect compared with an equimolar dose of mazindol. The *in vitro* inhibition of 5-HT uptake by forebrain synaptosomes isolated from rats pretreated *in vivo* with 35 μmol kg^{-1} of mazindol reached a peak 1 h after treatment and returned to normal within 10 h (Carruba & others, 1977). The inhibitory effect of mazindol on 5-HT uptake was not accompanied by a concomitant release of 5-HT from its storage depots (Carruba & others, 1977; Picotti & others, 1977). The present work is an extension of the above observation with the aim of further investigating the ability of mazindol to inhibit the 5-HT transport mechanism in rat brain synaptosomes *in vivo*. Experiments were made to determine whether mazindol might inhibit 5-HT depletion caused by the administration of *p*-chloromethamphetamine (pCMA), a drug which uses the 5-HT uptake pump to be transported into the neurons (Meek, Fuxe & Carlsson, 1971). The ability of mazindol to antagonize pCMA-induced 5-HT depletion was compared with that of chlorimipramine a drug already shown to be active at the dose used in counteracting the pCMA depleting action (Meek & others, 1971).

Albino Wistar male rats, Zambon S.p.A. Milan, 140–160 g, were injected intraperitoneally with pCMA (38 μmol kg^{-1}). Chlorimipramine HCl or mazindol (38 μmol kg^{-1}) were injected twice intraperitoneally (15 min and 2 h after injection of pCMA). The animals were decapitated 4 h later. The brains were quickly removed and frozen on dry ice. Spectrofluorimetric analyses for 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were made as described by Curzon & Green (1970). All groups consisted of 6 animals. Statistical comparisons among groups were made by the Dunnet test (Dunnet, 1964).

As shown in Table 1, pCMA provoked a marked decrease in brain 5-HT content 4 h after injection, while mazindol and chlorimipramine did not alter brain 5-HT concentrations. When mazindol and chlorimipramine were given in two doses after pCMA administration, the brain 5-HT depletion induced by pCMA was completely antagonized. Under these experimental conditions mazindol and chlorimipramine were equally potent in counteracting the action of pCMA.

Table 1. *Effect of mazindol and chlorimipramine on the depletion of brain 5-HT induced by* p-*chloromethamphetamine (pCMA) and on 5-HIAA brain concentrations.*

Treatment	Dose μmol kg^{-1}, ip	5-HT μg g^{-1} ± s.e.	5-HIAA μg g^{-1} ± s.e.
Saline	—	0·68 ± 0·01	0·42 ± 0·01
pCMA	38	0·43 ± 0·03*	
Mazindol	76	0·65 ± 0·01	0·43 ± 0·01
Chlorimipramine	76	0·70 ± 0·06	0·29 ± 0·01*
pCMA + mazindol	38 + 76	0·65 ± 0·03	
pCMA + chlorimipramine	38 + 76	0·67 ± 0·02	

* $P \leq 0{\cdot}01$ as compared to the saline treated animals. Each value is the mean ± s.e. of 6 determinations. Significance of difference among groups was calculated by the Dunnet test.

It has been suggested that chlorimipramine competitively antagonizes the active transport of pCMA into neurons via a membrane pump responsible for 5-HT uptake (Meek & others, 1971). Mazindol might be operating through a similar mechanism in this effect and it has been shown *in vitro* to inhibit competitively the active transport of 5-HT through the outer membranes of platelets (Picotti & others, 1977). Similar conclusions were reached by studying the inhibition of 5-HT uptake into synaptosomes obtained

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from the forebrains of mazindol-pretreated animals (Carruba & others, 1977).

Mazindol, however, seems to differ in another aspect from other 5-HT uptake inhibitors, such as imipramine (Bruinvels, 1972), Lilly 110140 (3-(*p*-trifluoromethylphenoxy)-*N*-methyl-3-phenyl-propylamine hydrochloride) (Fuller, Perry & Molloy, 1974) and chlorimipramine (Collard & Roberts 1974), in that it lacks their ability to decrease rat brain 5-HIAA concentrations. Brain 5-HIAA concentrations were not significantly different from those of the controls at any dose and any time studied. Doses of the drug of 8·7, 17·5 and 35 μmol kg^{-1} (s.c.) showed no significant variation in 5-HIAA content which ranged from 90·1 $\pm$ 5·5 to 107·5 $\pm$ 5·7 (% of controls) (Dunnet test) $n = 6$–10, control value 0·04 $\pm$ 0·02 μg g^{-1}. No change in the brain 5-HIAA content was detected by Garattini, Bizzi & others, (1975) 2 h after the injection of 52 μmol kg^{-1} of mazindol.

On the other hand, imipramine, Lilly 110140 and chlorimipramine have been reported to induce a decrease in brain 5-HIAA, probably through a decline in the 5-HT turnover rate which compensates for overstimulated receptor sites due to blockade of the reuptake of 5-HT at the nerve terminals (Da Prada & Pletscher, 1966; Corrodi & Fuxe, 1968; 1969; Fuller & others, 1974). The failure of mazindol to decrease the 5-HIAA brain concentrations, even when injected in high doses (Table 1), suggests that this drug operates in this area through a different mechanism which does not involve a compensatory reduction of 5-HT turnover rate. Previous data from this laboratory, showing that mazindol did not modify the pargyline-induced decline in 5-HIAA (Carruba, Groppetti & others, 1976) or the probenecid-induced accumulation of 5-HIAA (Carruba, Zambotti & others, 1975) in the rat brain, seem to support this hypothesis.

In conclusion our data show that mazindol, like chlorimipramine, inhibits 5-HT uptake *in vivo* but, unlike chlorimipramine it does not reduce the brain 5-HIAA concentrations.

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